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Does bovine respiratory disease treatment strategy influence chronic pneumonia and polyarthritis syndrome expression?

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Does Bovine Respiratory Disease Treatment Strategy Influence the Expression of Chronic Pneumonia and Polyarthritis Syndrome

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SASKATCHEWAN AGRICULTURE DEVELOPMENT FUND

Project Title

Does Bovine Respiratory Disease Treatment Strategy Influence the Expression of Chronic Pneumonia and Polyarthritis Syndrome?

Project Objectives (and sub-objectives)

The objective of this study was to determine if two particular management factors (injectable antimicrobial metaphylaxis at arrival to the feedlot and/or treatment management strategy) have an effect on the incidence of chronic pneumonia and polyarthritis syndrome (CPPS).

Additional objectives for this study included: 1) to describe the ecology of mycoplasma within pens of feedlot calves; 2) to determine if specific strains of *M. bovis* are related to pathogenicity in the feedlot, and 3) to describe the antibiotic sensitivity of the isolated organisms.

Project Accomplishments to date (Annual and Final Reports)

Mycoplasma pneumonia and arthritis has become one of the most frequently recognized causes of treatment and death in Canadian feedlots. Possible reasons to explain this increase in disease occurrence include: changes in the use of antibiotics to manage pneumonia in the feedlot (specifically, the use of antibiotics at arrival (metaphylaxis); and treating sick animals and immediately returning them back to their pen), co-infection with BVD virus, selective resistance to antibiotics, or a more severe disease causing strain.

The primary goal of this project was to investigate two management practices (metaphylaxis and treatment strategy) for their influence on the occurrence of Mycoplasma pneumonia and arthritis.

3,786 auctionmart-derived calves were placed into a southern Saskatchewan feedlot

during the fall of 2007 and 2008 (2007 - 1,751; 2008 – 2,035). All calves were vaccinated, dewormed and given a growth implant when processed at arrival, while only two-thirds of the calves received oxytetracycline metaphylaxis. Deep nasal swabs and ear notches were collected and frozen at processing. These calves were all co-mingled in 15 pens (7 pens in 2007; 8 pens in 2008).

As calves were pulled for initial pneumonia therapy, they were randomly assigned to one of two treatments: florfenicol given under the skin at label dose (6 ml/45 kg) and then returned home, or florfenicol given into the neck muscle at label dose (3 ml/45 kg) and then held in a "sick pen" for 48 hours prior to a second injection (3 ml/45 kg) before sending back to their home pen. At each pneumonia or arthritis treatment, a deep nasal swab was collected and frozen from each calf.

All calves with recurrent pneumonia were treated as per the feedlot's protocols. Any calves that died received a post-mortem examination. Swabs were collected from the lungs and stifle joint. These samples were collected and frozen at -20C.

Disease specific treatment and death rates were compared between the four groups of calves: 1) those receiving metaphylaxis and if treated, sent home immediately; 2) those receiving metaphylaxis and held for 48 hours before returning to home pen when treated; 3) those not receiving metaphylaxis, but sent home immediately if treated; and 4) those not receiving metaphylaxis and held for 48 hours before returning to home pen when treated.

The results of this study show that calves were 1.3 times more likely to be treated for pneumonia if they were not given oxytetracycline on arrival. This was the anticipated outcome based on previous research of giving antimicrobials at the time of arrival to the feedlot (Schumann et al., 1990; Van Donkersgoed, 1992). However, it is still surprising that oxytetracycline was relatively effective in controlling subsequent pneumonia in pens of comingled auction market-derived calves. No difference could be found between the 1st relapse rates for calves treated and sent home immediately as compared to treated and

held for 48 hours. The most likely explanation for this would be that relatively few animals were housed in the sick pen at any one time, which would minimize disease spread.

Treatment rates were lower than anticipated in both years of the study, resulting in very few relapse cases of pneumonia. Similarly, the death rate for pneumonia was not different between the four treatment groups. Not surprising then that the number of cases of Mycoplasma pneumonia and arthritis diagnosed at necropsy was quite low and not statistically different between our 4 groups.

The ear notches collected at processing on arrival from each calf were tested for the presence of BVD virus. Only one calf in 2007 and 3 calves in 2008 were positive. These calves did not increase the treatment rate in their respective pens. This may seem contradictory to what would be expected. However, we were not able to test all calves adjacent to our study pens which could have influenced our findings. That is, a persistently infected calf may have been in a pen adjacent to our study pens and increased the number of calves with pneumonia. We had 6 calves die with post-mortem findings of Mycoplasma pneumonia and arthritis, but none of them were persistently infected with BVD virus.

Deep nasal swabs were collected at processing from all calves, and from any animals that were treated or died. A select group of these swabs were submitted for culture of *Mycoplasma bovis*. As controls, calves that had been subsequently processed immediately after the above treated and/or dead calves had their swab from arrival also cultured. Only 23% of all the swabs cultured had *Mycoplasma bovis* isolated. The isolation rate was greatest from swabs collected at treatment and necropsy (48%) as compared to arrival (<1%). Given the retrospective selection of swabs to culture, it is quite plausible that more animals did have *Mycoplasma bovis* at arrival, but the storage (freeze-thaw) had decreased their viability. Other studies have also documented the low isolation rate of *M. bovis* from nasal swabs collected from cattle at arrival to the feedlot (Allen et al., 1991). However, by the end of the feeding period, many more animals will

have M. bovis present in their nasopharynx.

A total of 54 isolates of *Mycoplasma bovis* were cultured from the swabs and then evaluated using a DNA fingerprinting technique called Amplified Fragment Length Polymorphism (AFLP). It identified several clusters of *M. bovis* in the feedlot over the two years. The overall similarity of the isolates was 61.7%. As might be expected, the similarity was greatest for isolates collected from the same individual calves (87.3%). This was followed by the similarity of isolates collected within a pen (78.2%). Just as notable, 11 separate clusters of *M. bovis* were identified in the swabs collected at necropsy over the two years. It appears that Mycoplasma pneumonia and arthritis is not increasing in occurrence due to a particular disease causing strain.

Finally, the antimicrobial susceptibility of the same *M. bovis* isolates described above was determined for 10 common antimicrobials. As *M. bovis* lack a cell wall, antimicrobials such as ampicillin, and ceftiofur are of little efficacy and was apparent in the minimum inhibitory concentrations (MIC) measured. Similarly, *M. bovis* is inherently resistant to tilmicosin and erythromycin. The interpretation of the MIC is difficult given that there are no cut-off values available for *M. bovis* to determine resistance. However, when the MIC values are compared very little differences can be found between isolates, which suggests that antimicrobial resistance is likely not the cause for the increased occurrence of Mycoplasma pneumonia and arthritis.

The prevalence of *M. bovis* isolated from nasal deep nasal swabs or bronchoalveolar lavages increases during the feeding period (Allen et al., 1991). Given the lack of evidence for a particular disease-causing strain or antibiotic resistance, perhaps *M. bovis* is simply an opportunistic infection. Long-acting antimicrobials have significantly reduced the morbidity and mortality rates associated with shipping fever or Mannheimiosis. This means that more animals with acute pneumonia survive and perhaps the existing lung damage provides an adequate environment for Mycoplasma to become established.

Funding for this project has been graciously provided in equal amounts by the Beef Cattle Research Council, Ontario Cattlemen's Association, Agriculture Funding Consortium and Saskatchewan Agriculture Development Fund. A graduate student was not available for the completion of this project. However, it would have been very difficult to have a student completing course work and the daily tasks associated with this study. Instead, the student stipend supplement was put towards paying for extra labour required to process and treat calves, along with compensation to the feedlot clients for lost performance in the study animals.

The additional objectives added to this study are being achieved through funds generously provided by Intervet Schering-Plough Animal Health.

Detailed Accomplishments to date (Final Reports)

INTRODUCTION:

The respiratory tract of cattle is colonized by a wide variety of species of mycoplasma (bovis, dispar, bovirhinis, etc.). Fifteen to twenty years ago, isolation of *Mycoplasma bovis* from sick calves was considered to be an incidental finding. More recently, it has been associated with Chronic Pneumonia and Polyarthritis Syndrome (CPPS) seen in fall-placed feedlot calves. This syndrome has surpassed Mannheimiosis or shipping fever as the leading cause of mortality in feedlot calves in western Canada, accounting for 25-40% of calf death loss in these lots (Gagea et al., 2006). *Mycoplasma* respond very poorly to antimicrobial therapy (Ayling et al., 2006), which contributes to the chronicity of the disease. Calves with CPPS may live for several months. This not only creates an animal welfare issue for severely lame animals, but poor weight gains, aggressive antimicrobial therapy, poor carcass quality at slaughter, and increased labour demands, which all significantly add to the cost of the disease.

Several hypotheses have been proposed for the increasing incidence of CPPS in western Canadian feedlots, these include: 1) changes in the types and use of antibiotics to manage pneumonia in the feedlot, 2) a more severe disease causing strain of *Mycoplasma bovis*,

3) selective resistance to antibiotics, and 4) co-infection with BVD virus.

Introduction of long-acting antimicrobial therapy has changed the management of undifferentiated bovine respiratory disease (UBRD) in the feedlot. Calves are routinely given long-acting antimicrobials when entering the feedlot, termed metaphylaxis, as a preventative measure for UBRD. Metaphylaxis has been shown to reduce calf morbidity from UBRD, but not necessarily calf mortality, especially if measured beyond 28 days (Vandonkersgoed, 1992). This is in part due to more chronic cases of UBRD that die after the first month in the feedlot. Another change in the management of UBRD is that the temperatures of calves are no longer re-taken to objectively determine whether or not they have responded to antibiotic therapy. Instead calves are immediate returned to their home pen and are only re-evaluated if deemed sick by the feedlot personnel checking the pens. This too could lead to the development of more chronic cases of UBRD if calves in need of treatment are missed.

Mycoplasma bovis lacks a cell wall which means it is naturally resistant to several common antimicrobials (beta-lactams and cephalosporins), but at the same time is commonly found in the same lungs where antimicrobial susceptible pathogens such as *Mannheimia haemolytica* and *Pasteurella multocida* are found. It is quite plausible that antimicrobial resistance may serve as an important determinant in allowing *M. bovis* to develop an opportunistic infection. There are no minimum inhibitory concentration (MIC) cut-points determined by the Clinical and Laboratory Standards Institute for identifying resistant isolates of *M. bovis*. However, several papers have described the MIC for groups of isolates in Europe (Ayling et al., 2000), United States (Rosenbusch et al., 2005), Canada (Francoz et al., 2005), and Japan (Hirose et al., 2003).

Mycoplasma spp. can be recovered from a high percentage of calves within 2 weeks of arrival in the feedlot (Allen et al., 1991). However, it has not been well established if particular strains are more competitive and more pathogenic than others. This is an important question because it will impact the treatment and preventitive strategies used in the feedlot. The scientific literature contains a number of papers on how genetic

fingerprinting can be used in epidemiological studies to trace the spread of mycoplasma. However, these studies have been small in nature, involving fewer than a couple hundred animals; samples have come from passively obtained laboratory submissions versus from a controlled clinical field trial; and the majority of research has been conducted in Europe (McAuliffe et al., 2004; Kokotovic et al., 1999).

The immunosuppressive effects of bovine viral diarrhea virus (BVDV) on cattle has been well established. In particular, BVDV can significantly affect the morbidity and mortality rate of UBRD in pens with and adjacent to a persistently infected calf. However, there is conflicting evidence in the literature regarding the association between *M. bovis* and BVDV (Gagea et al., 2006; Shahriar et al., 2002). We speculate that given the multifactorial nature of CPPS, BVDV is most likely a component cause rather than a necessary cause.

This study will attempt to better understand the role of these several factors in a field study. The objectives of this project are to: 1) determine the effect of metaphylaxis and treatment strategy on the incidence of CPPS; 2) describe the ecology of mycoplasma within pens of feedlot calves; and 3) determine if specific strains or antibiotic resistance patterns of *M. bovis* are related to their pathogenicity in the feedlot; and 4) evaluate the association between CPPS and BVDV.

MATERIALS AND METHODS:

The study was conducted at a commercial feedlot located near Ceylon, Saskatchewan with a capacity of approximately 15,000 animals. The basic design of this feedlot is representative of standard designs used in western Canada. Open-air, dirt-floor pens are arranged side by side with central feed alleys. There are 60 large pens in the feedlot with capacities of 250 animals each. There is one hospital facility and one enclosed processing facility at the feedlot. Each facility has a hydraulic chute equipped with an individual animal scale, a chute-side computer for recording animal health data, and separation alleys to facilitate the return of animals to designated pens. In addition, there are three

recovery and "chronic" pens, 3 receiving pens, and several shipping pens located at the feedlot.

Study Animals

Animals enrolled in the study were crossbred beef steer and heifer calves purchased from auction markets throughout western Canada. Animals were transported by truck to the feedlot after assembly at auction markets. On arrival at the feedlot, animals were moved through a hydraulic chute for a group of procedures known collectively as processing. All animals were ear-tagged (to provide unique, individual animal identification, implanted with a trenbolone acetate and estradiol benzoate growth implant (Component EH/T or Component ES/T, Elanco Animal Health, Guelph, ON), and immunized with a multivalent clostridial bacterin–toxoid (Covexin 8, Intervet Schering- Plough Animal Health, Montreal, PQ). In addition, each animal received an infectious bovine rhinotracheitis virus, parainfluenza-3 virus, BVD virus, and bovine respiratory syncytial virus combination vaccine (Starvac 4, Novartis Animal Health), an *M. haemolytica* and *Pasteurella multocida* bacterin–toxoid (Somnustar PH, Novartis Animal Health), and topical ivermectin (0.5%; Vetomectin; ivermectin Pour-On, Durvet; 1.0 ml/10 kg). An ear notch and deep nasopharyngeal nasal swab was collected and frozen from each animal in the study. Samples were temporarily stored at the feedlot in a -20C freezer before being transported to the Western College of Veterinary Medicine where they were kept at -80C before testing. At an average days on feed (DOF) of approximately 100 days for each pen, all animals were re-implanted and vaccinated with an infectious bovine rhinotracheitis virus vaccine.

Experimental Design

A total of 3,786 animals arrived at the feedlot between October 26 and November 19, 2007; and October 22 and December 10, 2008. At processing animals were systematically randomized such that for every two calves given oxtetracycline metaphylaxis (**META**; n=2,512) (10 mg/kg SQ, Liquamycin LA-200, Pfizer Animal Health, Montreal, PQ), a third calf was not given metaphylaxis (**NO META**; n=1,274). After processing, animals were moved to designated feedlot pens. Each pen was checked once or twice daily by experienced animal health personnel for evidence of sickness.

Animals were deemed "sick" based on subjective criteria (e.g., general appearance and attitude, gauntness, reluctance to move) were moved from the feedlot pen and presented to one of the mobile hospital facilities. In this study, the case definition for initial UBRD was an elevated rectal temperature (≥40.0°C), an absence of abnormal clinical signs directly attributable to organ systems other than the respiratory system. All animals meeting the above case definition for UBRD where randomly allocated to one of two treatment strategies:

• **HOME** (n=1,889): Animals were treated in the neck region with a single dose of florfenicol (Nuflor) at 40 mg/kg SQ at the time of initial UBRD therapy.

• **HOLD** (n=1,897): Animals were treated in the neck region with a single dose of florfenicol (Nuflor) at 20 mg/kg IM at the time of initial UBRD therapy and were then held in a sick pen for 48 hours. After this time, calves had their temperature retaken and were administered a second dose of florfenicol (Nuflor) at 20 mg/kg IM. A maximum volume of 10 ml was administered at each injection site.

The animal health personnel were blinded to the experimental status of each animal. Animals selected as "sick" with recurrent clinical UBRD, based on the subjective assessment criteria described previously, were diagnosed as UBRD relapses provided there was an absence of abnormal clinical signs directly attributable to organ systems other than the respiratory tract and that at least 96 hours had elapsed since the most recent course of UBRD therapy. UBRD relapses were treated as follows, and all cattle were returned directly to their original feedlot pen after each treatment.

• **First UF relapse:** Single dose of enrofloxacin (Baytril 100, Bayer Animal Health; 10 mg/kg SQ in the neck region)

• **Second UF relapse:** three doses of trimethoprim-sulfadoxine (Trivetrin, Intervet Schering-Plough Animal Health; 16 mg/kg IM in the neck region)

Two post-allocation relapse treatment regimes (first and second UBRD relapses) were the maximum permitted for all animals in the study. Once an animal was treated as a second UBRD relapse, no further treatment for UBRD was attempted. Animals that were identified with clinical UBRD subsequent to treatment for a third UBRD relapse were deemed to be "chronics," as were animals unsuitable to be returned to their designated

feedlot pens after treatment based on subjective appraisal of the attitude and appearance. Finally, all other diseases were treated according to a standard feedlot protocol provided by consulting veterinarians. All treatment events, including treatment date, presumptive diagnosis, and drug(s) and dose(s) administered, were recorded in the chute-side computer system. The animal health events of each animal were followed from allocation to re-implantation (100 DOF). Feedlot veterinarians prosected all animals that died during the study using a standardized method and collected lung and joint swabs as outlined in the written necropsy protocol provided by the study investigators.

Based on the 2x2 factorial design the four experimental groups included: 1) mass medicated, treat and send to home pen (**META-HOME**), mass medicated, treat and hold in sick pen for 48 hours (**META-HOLD**), no-metaphylaxis, treat and send to home pen (**NO META-HOME**), and no-metaphylaxis, treat and hold in sick pen for 48 hours (**NO META-HOLD**).

Feeding Program

Standard mixed complete feedlot diets, formulated to meet or exceed National Research Council nutritional requirements of feedlot cattle were offered ad libitum. Feedlot diets were blended by combining dry-rolled barley, barley silage, alfalfa hay, and supplement in a modern, batch-milling facility equipped with overhead bins. Animals were adapted to a backgrounding diet over a 30-day period by increasing the proportion of dry-rolled barley and decreasing the proportions of barley silage and alfalfa hay at approximately 7-day intervals. Diets were delivered to pens once or twice daily using truck-mounted mixers on load cells. Daily feed allowances to each pen were recorded. Water was provided ad libitum.

Data Collection and Management

The computerized animal health data were collected, verified, and summarized. From this data, risk rates for initial UBRD, first UBRD relapse, second UBRD relapse, overall mortality (mortality due to all causes), UBRD mortality (mortality due to UBRD), histophilosis mortality (mortality due to *H. somni* infection), lameness mortality

(mortality due to lameness), BVD/enteritis mortality (mortality due to BVD or enteritis), metabolic mortality (mortality due to metabolic disease), and other mortality (mortality due to causes other than UBRD, histophilosis, lameness, BVD/enteritis, or metabolic disease) were calculated for each experimental group. The weight of each animal was measured and recorded at arrival, and re-implantation. These data were subsequently imported into a spreadsheet program (Microsoft Excel 2003), where the ancillary production and feedlot performance variables (arrival weight, re-implantation weight, weight gain, and ADG) were calculated for each animal and summarized for each experimental group.

Statistical Analysis

Data were analyzed using an analytical software program (SAS for Windows, Release 9.1, SAS Institute, Cary, NC). The various animal health indices were compared between the experimental groups using logistic regression in a log linear model for experimental group effects, with individual animals as the unit of analysis. Differences were deemed significant when $P < 0.05$. Calculation of 95% confidence intervals was done using the partially maximized likelihood function (likelihood ratio-based confidence intervals). Ancillary production and performance variables were compared between the experimental groups using least squares analysis of variance for experimental group effects. The baseline variables were tested as covariates of the feedlot performance variables and included in the final model used for comparison of each variable between the experimental groups when significant ($P < 0.05$) effects were detected.

Selection of Samples for Mycoplasma Isolation:

Upon completion of the project, all samples collected were reviewed for completeness. Calves that had died after 20 days in the feedlot had their corresponding nasal, lung and joint swabs cultured for *M. bovis* at the Animal Health Laboratory (University of Guelph, Guelph, ON) on modified Hayflicks media for 3 to 10 days at 37C with 5% CO_2. A random sampling of calves that were treated for UBRD, but survived the feeding period had their nasal swabs from treatment and arrival submitted for mycoplasma isolation. For each of the above calves, a "control" animal was selected. These control animals

were the next animal processed at arrival that remained healthy (i.e. no treatment) during the entire backgrounding period. The nasal swab collected at induction to the feedlot was then submitted for culture.

AFLP Methods and Analysis

Mycoplasma cells were collected from 5-ml cultures by centrifugation at 13,000 x g for 10 minutes, and the genomic DNA was extracted from the cell pellet using the DNeasy tissue kit (QIAGEN, Mississauga, ON) following the manufacturer's protocol for Gram-positive bacteria. The procedures used for Amplified Fragment Length Polymorphism (AFLP) analysis were as described by Kokotovic et al. (1999). Briefly, the genomic DNA was digested with restriction enzymes *Bgl*II and *Mfe*I, and the resulting DNA fragments were ligated to specific adapters. The *Bgl*II-*Mfe*I fragments tagged with specific adapters were then amplified by PCR using *Bgl*II-0 (labeled with FAM) and *Mfe*I-0 primers. The sequences of the adapters and primers and PCR conditions were as described previously (Kokotovic et al., 1999). The amplified DNA fragments were separated using ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). The GS LIZ 600 size standard (Applied Biosystems) was used as an internal size standard for each sample. The fragment data were analyzed for size calling and intensity normalization by using the GeneMapper v4.0 software (Applied Biosystems). All electropherograms were also visually inspected to ensure data quality. Fragments in the range from 60 to 600 bp were included in the analysis because of high data quality in this range.

Further data analysis for determining the relationship of various samples was carried out using the BioNumerics v5.1 software (Applied Maths, Austin, TX). The files generated using the GeneMapper software were inputted into the BioNumerics software, and the fragments were compared using the Band Matching function of the software with optimal settings as verified by visual inspection . Uncertain bands were excluded in the analysis by using the software's Uncertain Bands function. The similarities among the fingerprints of each sample were calculated using the Binary Dice coefficient. Dendrograms were constructed by using Unweighted Pair Group Method using Arithmetic averages (UPGMA). The cluster grouping was based on a 95% similarity of the fingerprints. The

AFLP fingerprints of representative groups were visually examined to ensure that the software data analysis outcomes also matched with the visual pattern determination.

Minimum Inhibitory Concentration (MIC)

Isolates were tested for antimicrobial activity using Sensititre plates custom prepared (Nova Century Scientific Inc., Burlington, Ontario) by drying doubling dilutions of antimicrobials on round-bottom 96-well plates. Ten different antimicrobials were tested, including ampicillin, chlortetracycline, ceftiofur, enrofloxacin, erythromycin, florfenicol, oxytetracycline, spectinomycin, tilmicosin and tulathromycin. The cell wall active antimicrobials ampicillin and ceftiofur were not expected to show activity against a mycoplasma, and they were included in this study as negative controls. Test dilutions were 0.25–32 mg/ml for ampicillin; 0.06-32 mg/ml for erythromycin and florfenicol; 0.03–32 mg/ml for chlortetracycline and oxytetracycline; 0.125–64 mg/ml for ceftiofur; 0.03–4 mg/ml for enrofloxacin; 0.12–256 mg/ml for spectinomycin; 0.5–128 mg/ml for tilmicosin; and 1-32 mg/ml for tulathromycin. A standard dilution of culture was added to each well so that $2x10^3$ to $2x10^5$ colour-changing units per well were delivered in a final 200-ml volume. Ten-fold dilutions of the challenge culture were also plated into wells without antimicrobial to confirm that the challenge was within the acceptable range of color-changing units per well. After sealing the plates, they were incubated at 37C in a 5% CO_2 atmosphere for 48 hr. The lowest concentration of antimicrobial suppressing growth, as expressed by blue to red shift, was recorded as the minimum inhibitory concentration (MIC) for that antimicrobial.

Immunohistochemistry for BVDV

All ear notches were collected at arrival to the feedlot and frozen at -20°C until tested for BVD virus using immunohistochemistry at Prairie Diagnostic Services, Saskatoon, SK. Ear notches were fixed in 10% buffered formalin solution for a minimum of 24–48 hr and embedded in paraffin within 1 week. Paraffin-embedded skin was serially sectioned, mounted on poly-L-lysine–coated slides, and stained for BVDV using monoclonal antibody 15C5. Diaminobenzidine (DAB) served as the chromogen. Positive controls

stained concurrently with each batch were tissues from cattle that died from mucosal disease. Each section was then examined for the presence or absence of BVDV staining.

RESULTS:

The calves in this study weighed 247 kg (95% CI: 245-249 kg) on arrival to the feedlot. At the completion of their backgrounding phase, an average of 102 days on feed, they weighed an average of 356 kg (95% CI: 354-358 kg). This gave them an average daily gain (ADG) of 1.08 kg/day (95% CI: 1.06-1.10 kg/day). Table 1 summarizes the ADG for the 4 treatment groups. Calves that were given metaphylaxis and held in a sick pen for 48 hours, had a significantly lower ADG than calves not given metaphylaxis, but treated and returned to their home pen.

The morbidity and mortality results are summarized in Tables 2 through 7 for each of the two study years separately and combined. The only significant difference found was that calves receiving metaphylaxis are less likely (relative risk=0.77) to be treated for UBRD as compared to calves not given metaphylaxis ($P<0.05$). No differences were found in relapse or mortality rates between the four treatment groups for either of the years. In the first year of data (2007), their was a tendency for calves given metaphylaxis to be treated more for arthritis than calves not given metaphylaxis on arrival to the feedlot ($P=0.10$). However, this association was not present in the 2008 data or when all data was combined. UBRD remains the number one cause of mortality for feedlot calves.

In 2007, a total of 16 dead calves had 49 corresponding swab samples from arrival, treatment and post-mortem cultured for *M. bovis*, 14/20 (70%) post-mortem lung and joint swabs were positive; 0/16 (0%) swabs collected at arrival were positive; 8/13 (62%) treatment swabs from the dead calves were positive. Another 16 calves treated for UBRD, but survived, had 40 corresponding samples cultured (0/16 (0%) nasal swabs from arrival were positive; 7/24 (29%) nasal swabs from treatment were positive). A total of 32 healthy "control" calves had their nasal swab collected at arrival to the feedlot cultured for mycoplasma (2/32 (6%) of these samples were positive for *M. bovis*.

Similarly in 2008, 13 dead calves resulted in 38 samples for *M. bovis* isolation (7/13 (54%) positive cultures from post-mortem swabs; 6/12 (50%) positive treatment swabs; 0/13 (0%) positive swabs from arrival). There were 16 calves treated for UBRD that survived the feeding period with a total of 44 samples, 8/28 (29%) of the swabs collected at treatment had *M. bovis* isolated, while 0/16 swabs from arrival were positive. A total of 29 control calves were selected and 0/29 of their swabs from arrival were positive for *M. bovis*. In total, 31 strains of *M. bovis* were isolated in 2007 out of 121 cultures giving an isolation rate of 26%. Twenty three strains were cultured in 2008 out of 111 samples resulting in an isolation rate of 21%. Dendrograms summarizing the genetic similarity between strains collected in 2007, 2008 and combined years are included in Figures 1 to 3. Based on 95% similarity, 13 clusters were identified in 2007, 10 clusters in 2008 and 20 clusters from both years combined. Similar dendrograms were constructed to evaluate the similarity between strains collected exclusively at post-mortem (Figures 4 to 6).

The Dice similarity coefficients were summarized for groups of strains within a calf, pen or year to see where the greatest or least amount of variability in strains exist (Tables 8 and 9). The CCIA numbers for positive calves was compared to see if *M. bovis* was clustering by herd of origin.

Strains were subjected to MIC determination for 10 common antimicrobials used in food animal production and the results are summarized by study year in Tables 10 and 11. There is very little variability in the MIC values for particular antimicrobials. Small changes in MIC can be noticed within isolates from the same calf, but not always inferring more resistance. All M. bovis strains had MIC values greater than or equal to the maximum concentration evaluated for ampicillin, ceftiofur, erythromycin and tilmicosin.

The ear notches collected at induction were tested for the presence of BVD virus using immunohistochemistry. Only one calf in 2007 and 3 calves in 2008 tested positive. The pen-level morbidity and mortality rates with direct BVDV exposure are summarized in Table 12.

DISCUSSION:

The results of this study show that calves were less likely (0.77 times) to be treated for pneumonia if they were given oxytetracycline at induction. This was the anticipated outcome based on previous research of giving antimicrobials at the time of arrival to the feedlot (Schumann et al., 1990; Van Donkersgoed, 1992). Many feedlots in recent years have tend to use antimicrobials such as tulathromycin or tilmicosin for metaphylaxis, however, it is interesting to see that oxytetracycline was relatively effective in controlling subsequent pneumonia in pens of comingled auction market-derived calves. Contrary to the above findings, metaphylaxis appears to decrease the ADG during the first 100 DOF. This finding is opposite to what has been previously reported in the literature (VanDonkersgoed, 1992). We are unclear as to how to explain this result.

No difference could be found between the first relapse rates for calves treated and sent home immediately as compared to treated and held for 48 hours. However, we did find that calves held in a sick pen for 48 hours did have a lower ADG during the first 100 days in the feedlot as compared to calves that were returned to their home pen. Despite the relatively few animals that were housed in the sick pen at any one time, further disease transmission between sick calves could be quite plausible as the cause for this.

We would have preferred cattle of high risk of developing UBRD for this study, as treatment rates of less than 10% would suggest that these calves were of low to moderate risk. With treatment rates lower than anticipated in both years of the study, there were very few UBRD mortalities and cases of CPPS. No statistical differences could be found between either of the 4 treatment groups in these two outcome measures. Our results would suggest that both metaphylaxis and UBRD treatment strategy do not impact the occurrence of CPPS, but we must be cautious in this interpretation given the low power of this study. We had based our sample size on higher UBRD treatment rates from previous years in this feedlot. It is interesting to note that during the years of this study, cattle were brought into the feedlot over a longer period of time (September to February),

which is quite different from the past (October to December).

BVDV persistently infected calves did not increase the treatment rate in their respective pens. This may seem contradictory to what has previously been reported in the literature (Booker et al., 2005). However, we were not able to test all calves adjacent to our study pens which may have influenced our findings. We had 6 calves die with signs of Mycoplasma pneumonia and arthritis on post-mortem examination, but none of them were persistently infected with BVD virus.

Only 22% of all the swabs cultured had *Mycoplasma bovis* isolated. The isolation rate was greatest from swabs collected at treatment and necropsy (45%) as compared to arrival (<1%). Given the retrospective selection of swabs to culture, it is quite plausible that more animals did have *Mycoplasma bovis* at arrival, but the freeze thaw associated with transport and storage decreased their viability. Other studies have also documented low isolation rates of *M. bovis* from nasal swabs collected from cattle at arrival to the feedlot. Our concern is the bias introduced into the cluster analysis given that most of the isolates were actually from animals with UBRD and only two strains from healthy animals at arrival. Serial sampling of cattle throughout the feeding period might reveal some further interesting findings.

Twenty clusters of *M. bovis* were identified in the feedlot over the two years from 54 strains. The overall similarity of the isolates was 61.7%. As might be expected, the similarity was greatest for isolates collected from the same individual calves (87.3%). This was followed by the similarity of isolates collected within a pen (78.2%). Just as notable, 11 separate clusters of *M. bovis* were identified in the swabs collected at necropsy over the two years. Based on the above findings, we believe that CPPS is not increasing in occurrence due to a particular disease causing strain. McAuliffe et al. (2004) describe two groups of isolates (A and B) with two subgroups within B (Ba and Bb). These two groupings were not as distinct in these groups of isolates. The variability in isolates as demonstrated by the large number of clusters is not surprising given how the cattle in this study were procured. Ribble et al. (1994) demonstrated that in each

feedlot pen of 200 calves, approximately 60 different farms of origin are represented. This means that there is an incredible amount of mixing in a feedlot and auction market. When we examined the Canadian Cattle Identification Agency number for each calf, we found that on average 3 to 4 calves had a similar sequenced tag and entered the feedlot at the same time. This would confirm the same degree of mixing in these calves. Future research should look at the isolation of M. bovis from cows and calves on ranches to determine its prevalence and degree of similarity within and between farms.

The antimicrobial susceptibility of the *M. bovis* isolates was determined for 10 common antimicrobials (Tables 10 and 11). The interpretation of these minimum inhibitory concentrations (MIC) is difficult given that there are no cut-off values to determine resistance. However, when the MIC values are compared very little differences can be found between isolates, which suggests that antimicrobial resistance is also not the cause for the increased occurrence of Mycoplasma pneumonia and arthritis. The greatest proportions of these isolates were collected from cattle with UBRD, so it is quite plausible that they are quite similar, but still different than *M. bovis* from untreated cattle.

The prevalence of *M. bovis* isolated from nasal deep nasal swabs or bronchoalveolar lavages increases during the feeding period (Allen et al., 1991). Long-acting antimicrobials have significantly reduced the morbidity and mortality rates associated with shipping fever or Mannheimiosis. This means that more animals with acute pneumonia survive and perhaps the existing lung damage provides an adequate environment for Mycoplasma to become established. Given the lack of evidence for a particular disease-causing strain or antibiotic resistance, it would seem prudent to further evaluate the differences in virulence factors between isolates. Although AFLP has been found to discriminate well between strains of *M. bovis*, we can not rule out the possibility that there are virulence factor differences between these strains.

CONCLUSION:

In conclusion, this field study has provided a unique opportunity to gain new insight on the epidemiology of *M. bovis*. We can not rule out the possibility that UBRD

management could still play a role in the development of CPPS, but the presence of more virulent strains or antimicrobial resistance is less likely than we thought. BVDV remains an important immunosuppressant and component cause of UBRD, but is unlikely the cause for increasing incidence of CPPS in western Canadian feedlots.

Project Milestones and Changes from Original Proposal and Reports

Milestone	Description	Proposed Dates	Modified Dates
Milestone 1	Enrolment of Calves into the Study	Sep 07 to Dec 07	Oct 07 to Nov 08
Milestone 2	Completion of Data Collection	Sep 07 to Jul 08	Oct 07 to Sep 09
Milestone 3	Interim Report to ADF	Sep 07 to Sep 08	Sep 07 to Sep 08
Milestone 4	Analysis of Data and Preparation of Manuscripts	Sep 08 to Mar 09	Jul 08 to Nov 09
Milestone 5	Final Report to ADF	Sep 08 to Sep 09	Sep 08 to Dec 09

The milestone dates have been modified from our original proposal as the data collection for this project was be completed over two falls instead of just one. This was a positive change because it allowed us to see if the same treatment effects were different over two years of sample collection. The disease risks faced in the feedlot are often quite different each fall depending on the weather and the rate at which the feedlot fills.

Technology Transfer

This data has been reported to a group of Saskatchewan veterinarians, but further dissemination is planned in the near future.

List of Publications

We have no publications at this time, although two manuscripts have been prepared for publication.

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VanDonkersgoed J. 1992. Meta-analysis of field trial of antimicrobial mass medication for prophylaxis of bovine respiratory disease in feedlot cattle. Can Vet J 33:786-795.

Table 1: A summary of the average daily gain (kg/day and 95% confidence interval) for calves treated for UBRD in the four treatment groups.

Treatment	ADG (kg/day)	95% CI (kg/day)	P-value
Meta-Hold	0.83	0.73-0.94	<0.01
Meta-Home	0.95	0.85-1.06	0.12
No Meta-Hold	0.96	0.83-1.10	0.17
No Meta-Home	1.09	0.94-1.24	Ref

Table 2: Morbidity data for a total of 1751 cross-bred auction mart derived calves placed into 7 feedlot pens in a southern Saskatchewan feedlot during the fall of 2007. Calves were randomized to one of four treatment groups.

Morbidity	Metaphylaxis		No Metaphylaxis		Total:
	Home	Hold	Home	Hold	
N	579	582	296	294	1751
1st BRD Treatment	39 6.7%[a]	37 6.4%[a]	34 11.5%[b]	22 7.5%[b]	132 7.5%
1st BRD Relapse	2 5.1%[a]	2 5.4%[a]	1 2.9%[a]	2 9.0%[a]	7 5.3%
1st Arthritis Treatment	13 2.2%[a]	14 2.4%[a]	3 1.0%[a]	2 0.7%[a]	32 1.8%

[a,b] – signifcantly different at $P<0.05$ within each row

Table 3: Morbidity data for a total of 2035 cross-bred auction mart derived calves placed into 8 feedlot pens in a southern Saskatchewan feedlot during the fall of 2008. Calves were randomized to one of four treatment groups.

Morbidity	Metaphylaxis		No Metaphylaxis		Total:
	Home	Hold	Home	Hold	
N	676	675	338	346	2035
1st BRD Treatment	42 6.2%[a]	42 6.2%[a]	27 7.9%[b]	26 7.5%[b]	137 6.7%
1st BRD Relapse	3 7.1%[a]	2 4.8%[a]	1 3.7%[a]	1 3.8%[a]	7 5.1%
1st Arthritis Treatment	4 0.6%[a]	9 0.1%[a]	2 0.6%[a]	4 1.2%[a]	19 0.9%

[a,b] – signifcantly different at $P<0.05$ within each row

Table 4: Morbidity data for a total of 3,786 cross-bred auction mart derived calves placed into 15 feedlot pens in a southern Saskatchewan feedlot during the fall of 2007 and 2008. Calves were randomized to one of four treatment groups.

Morbidity	Metaphylaxis		No Metaphylaxis		Total:
	Home	Hold	Home	Hold	
N	1255	1257	634	640	3786
1st BRD Treatment	81 6.5%[a]	79 6.3%[a]	61 9.6%[b]	48 7.5%[b]	269 7.1%
1st BRD Relapse	5 6.2%[a]	4 5.1%[a]	2 3.3%[a]	3 6.3%[a]	14 5.2%
1st Arthritis Treatment	17 1.4%[a]	23 1.8%[a]	5 0.8%[a]	6 0.9%[a]	51 0.9%

[a,b] – signifcantly different at $P<0.05$ within each row

Table 5: Mortality data for a total of 1751 cross-bred auction mart derived calves placed into 7 feedlot pens in a southern Saskatchewan feedlot during the fall of 2007. Calves were randomized to one of four treatment groups.

Mortality	Metaphylaxis		No Metaphylaxis		Total
	Home	Hold	Home	Hold	
N	579	582	296	294	1751
Overall	13 2.2%	11 1.9%	4 1.4%	6 2.0%	34 1.9%
BRD	10 77%	8 73%	2 50%	4 67%	24 71%
CPPS	0 0%	1 9%	1 25%	2 33%	4 12%
Histophilosis	1 8%	2 18%	1 25%	0 0%	4 12%
Misc.	2 15%	0 0%	0 0%	0 0%	2 5%

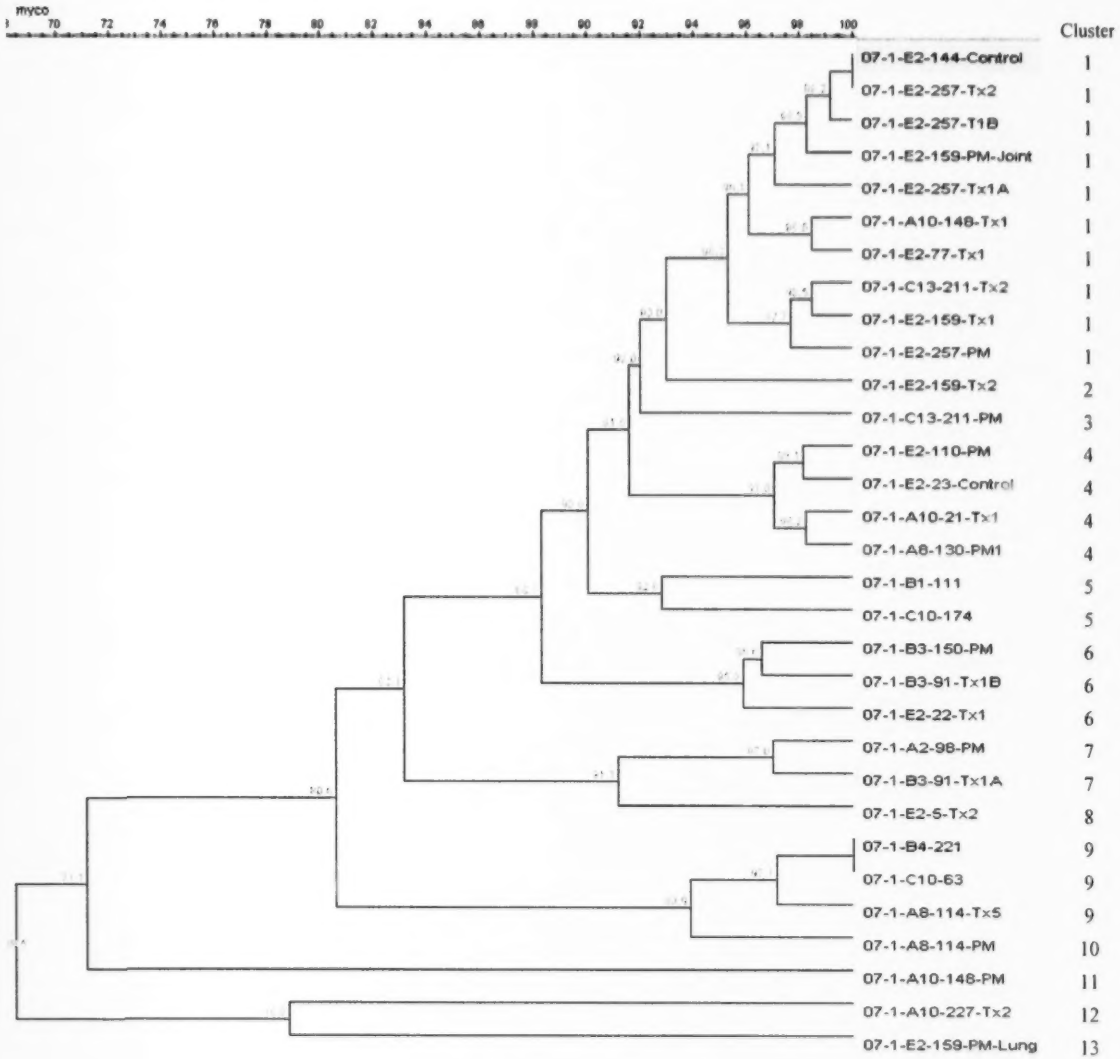
Table 6: Mortality data for a total of 2035 cross-bred auction mart derived calves placed into 8 feedlot pens in a southern Saskatchewan feedlot during the fall of 2008. Calves were randomized to one of four treatment groups.

Mortality	Metaphylaxis		No Metaphylaxis		Total
	Home	Hold	Home	Hold	
N	676	675	338	346	2035
Overall	12 1.8%	4 0.6%	7 2.1%	10 2.9%	33 1.6%
BRD	9 75%	2 50%	5 71%	7 70%	23 70%
CPPS	0 0%	1 25%	0 0%	1 10%	2 6%
Histophilosis	1 8%	0 0%	0 0%	0 0%	1 3%
Misc.	2 15%	1 25%	2 29%	2 20%	7 21%

Table 7: Mortality data for a total of 3,786 cross-bred auction mart derived calves placed into 15 feedlot pens in a southern Saskatchewan feedlot during the fall of 2007. Calves were randomized to one of four treatment groups.

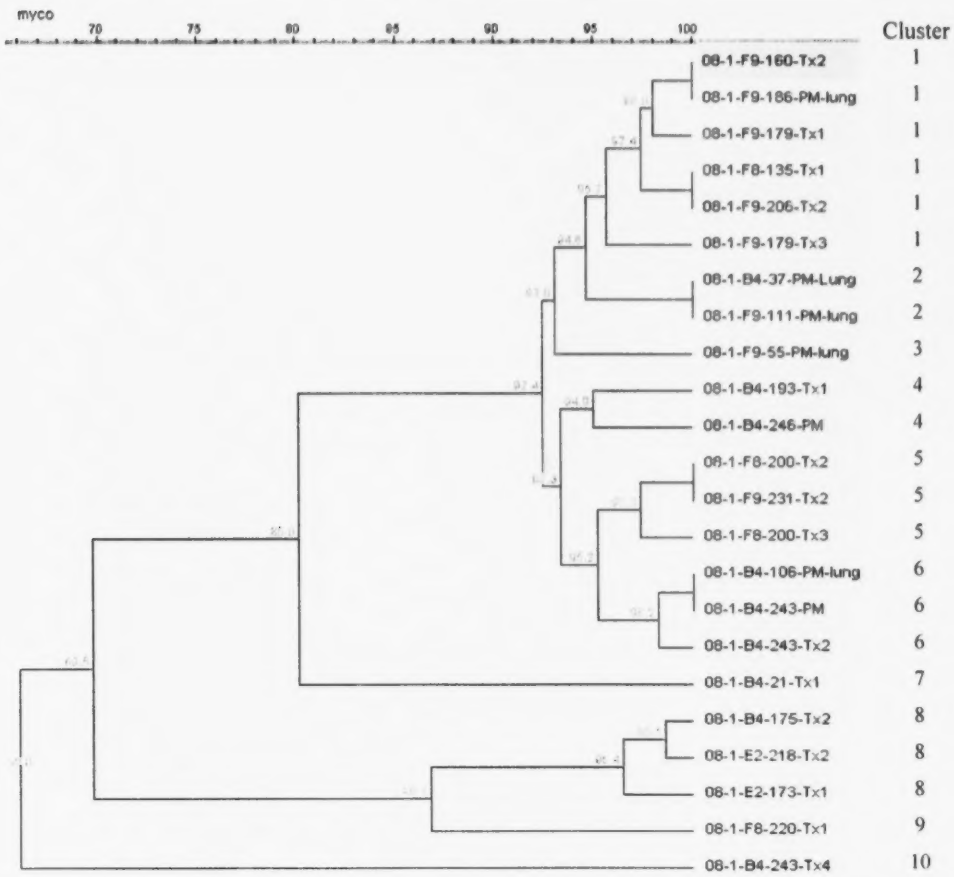
Mortality	Metaphylaxis		No Metaphylaxis		Total
	Home	Hold	Home	Hold	
N	1255	1257	634	640	3786
Overall	25	15	11	16	67
	2.0%	1.2%	1.7%	2.5%	1.8%
BRD	19	10	7	11	47
	76%	67%	64%	69%	70%
CPPS	0	2	1	3	6
	0%	13%	9%	19%	9%
Histophilosis	2	2	1	0	5
	8%	13%	9%	0%	7.5%
Misc.	4	1	2	2	9
	16%	6.6%	18%	12.5%	13%

Figure 1: Genetic relationship between *M. bovis* strains collected in 2007 based on comparison of AFLP profiles (60- to 600-bp fragment size range) produced by amplification of *Bgl*II and *Mfe*I DNA templates with non-selective primers. The dendrogram demonstrating was produced with the UPGMA method with Dice similarity coefficient (S_D). Cluster represents the strains with 95% similarity.



07, year
1, feedlot identification
E2, pen identification
159, calf identification
PM, post-mortem or treatment number (Tx1A, Tx1B, Tx2, etc)
Lung, tissue sampled (lung, joint, nasal swab is the default if not indicated otherwise)

Figure 2: Genetic relationship between *M. bovis* strains collected in 2008 based on comparison of AFLP profiles (60- to 600-bp fragment size range) produced by amplification of *Bgl*II and *Mfe*I DNA templates with non-selective primers. The dendrogram demonstrating was produced with the UPGMA method with Dice similarity coefficient (S_D). Cluster represents the strains with 95% similarity.



08, year
1, feedlot identification
B4, pen identification
243, calf identification
Tx4, post-mortem or treatment number (Tx1A, Tx1B, Tx2, etc)
Tissue sampled (lung, joint, nasal swab is the default if not indicated otherwise)

Figure 3: Genetic relationship between *M. bovis* strains collected in 2007 and 2008 based on comparison of AFLP profiles (60- to 600-bp fragment size range) produced by amplification of *Bgl*II and *Mfe*I DNA templates with non-selective primers. The dendrogram demonstrating was produced with the UPGMA method with Dice similarity coefficient (S_D). Cluster represents the strains with 95% similarity.

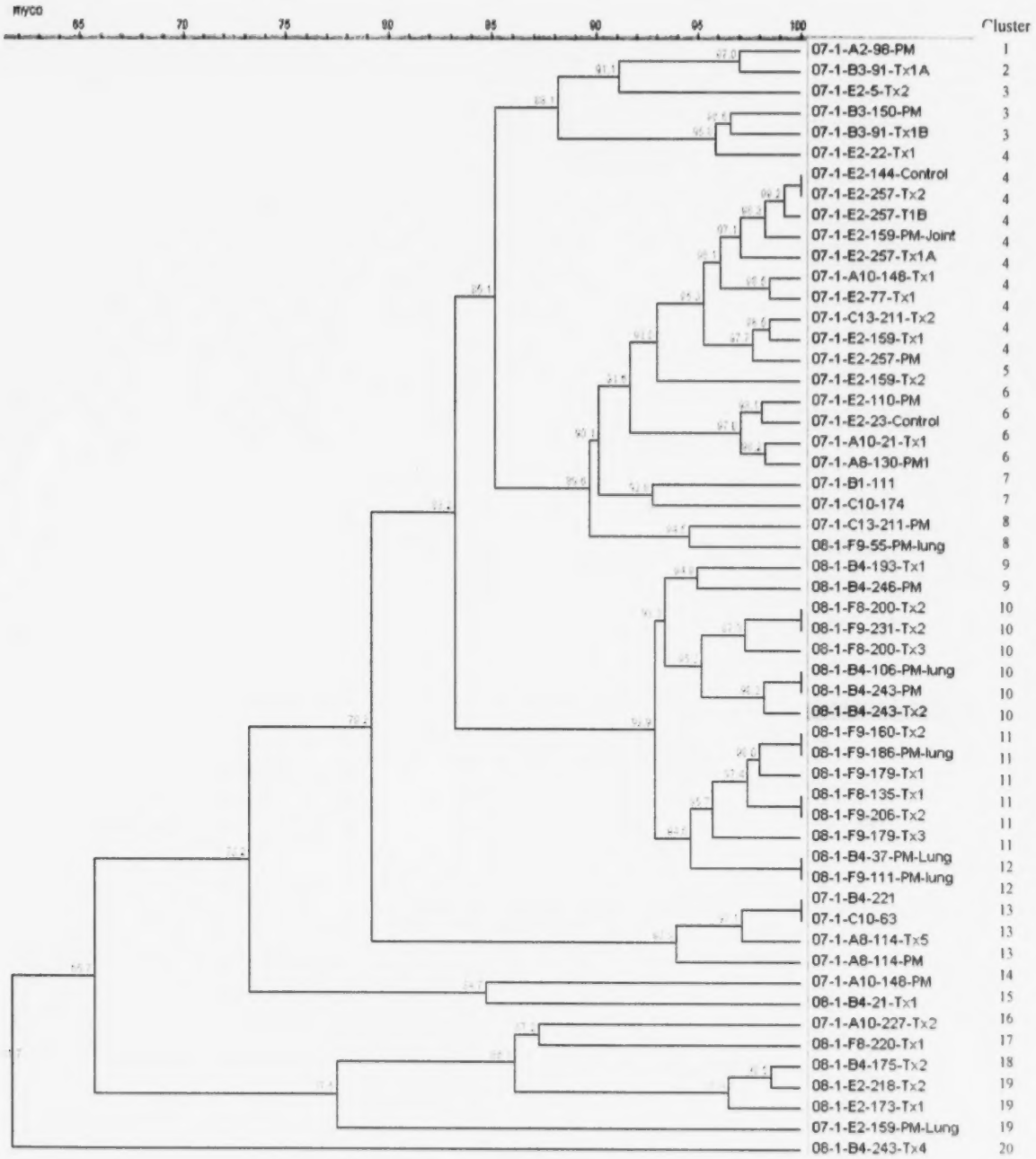


Figure 4: Genetic relationship between *M. bovis* strains collected exclusively at post-mortem in 2007 based on the comparison of AFLP profiles (60- to 600-bp fragment size range) produced by amplification of *Bgl*II and *Mfe*I DNA templates with non-selective primers. The dendrogram demonstrating was produced with the UPGMA method with Dice similarity coefficient (S_D). Cluster represents the strains with 95% similarity.

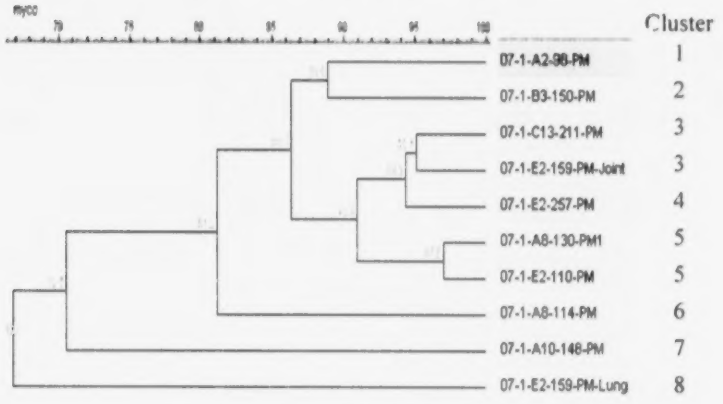


Figure 5: Genetic relationship between *M. bovis* strains collected exclusively at post-mortem in 2008 based on comparison of AFLP profiles (60- to 600-bp fragment size range) produced by amplification of *Bgl*II and *Mfe*I DNA templates with non-selective primers. The dendrogram demonstrating was produced with the UPGMA method with Dice similarity coefficient (S_D). Cluster represents the strains with 95% similarity.

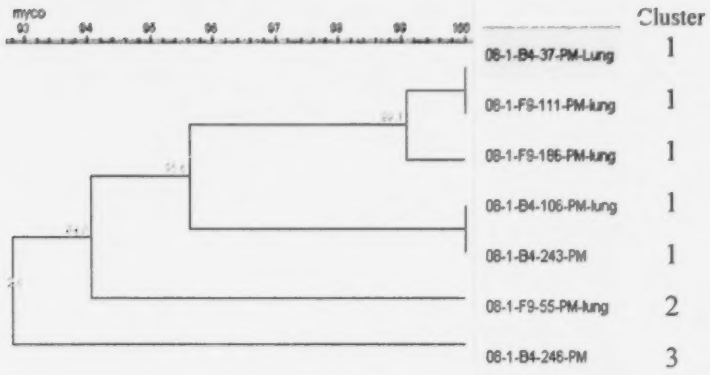


Figure 6: Genetic relationship between *M. bovis* strains collected at post-mortem in 2007 and 2008 based on comparison of AFLP profiles (60- to 600-bp fragment size range) produced by amplification of *Bgl*II and *Mfe*I DNA templates with non-selective primers. The dendrogram demonstrating was produced with the UPGMA method with Dice similarity coefficient (S_D). Cluster represents the strains with 95% similarity.

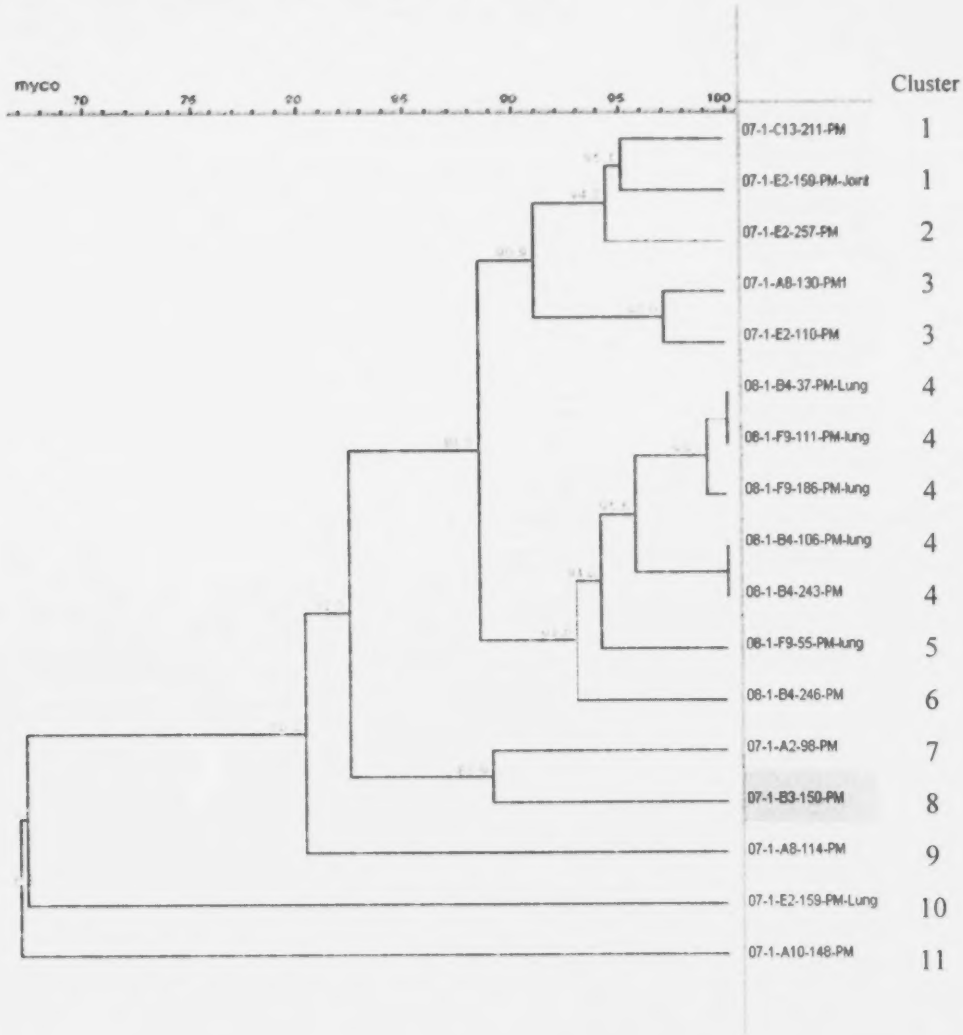


Table 8: Summary of mean Dice similarity coefficients between strains isolated within a calf, pen or feedlot for samples collected in 2007.

Strain ID	CCIA ID (124 256 084 XXX)	Similarity of isolates within a calf	Similarity of isolates within a pen	Similarity of isolates within the feedlot (year)
07-1-A10-21-Tx1	256084	N/A	63.9%	66.5%
07-1-A10-148-Tx1 07-1-A10-148-PM-lung	257849	80%		
07-1-A10-227-Tx2	261734	N/A		
07-1-A2-98-PM-lung	257548	N/A	N/A	
07-1-A8-114-Tx5 07-1-A8-114-PM-lung	258306	96.5%	86.1%	
07-1-A8-130-PM-joint 07-1-A8-130-PM-lung	260836	92%		
07-1-B3-91-Tx1A 07-1-B3-91-Tx1B	257690	90.6%	90.2%	
07-1-B3-150-PM-lung	256328	N/A		
07-1-C13-211-Tx2 07-1-C13-211-PM-lung	256316	90.3%	90.3%	
07-1-E2-5-Tx2	259567	N/A	70.4%	
07-1-E2-22-Tx1	259605	N/A		
07-1-E2-23-Control	263001	N/A		
07-1-E2-77-Tx1	259574	N/A		
07-1-E2-110-PM-lung	285238	N/A		
07-1-E2-144-Control	257038	N/A		
07-1-E2-159-Tx1A 07-1-E2-159-Tx1B 07-1-E2-159-PM-joint 07-1-E2-159-PM-lung	**278279**	70.6%		
07-1-E2-257-Tx1A 07-1-E2-257-Tx1B 07-1-E2-257-Tx2 07-1-E2-257-PM-lung	**278279**	95.7%		

Table 9: Summary of mean Dice similarity coefficients between strains isolated within a calf, pen or feedlot for samples collected in 2008.

Strain ID	CCIA ID (124 256 084 XXX)	Similarity of isolates within a calf	Similarity of isolates within a pen	Similarity of isolates within the feedlot (year)
08-1-B4-21-Tx1	**253125**	N/A	68.6%	66.0%
08-1-B4-37-PM-lung	251433	N/A		
08-1-B4-106-PM-lung	252215	N/A		
08-1-B4-175-Tx2	**253125**	N/A		
08-1-B4-193-Tx1	252235	N/A		
08-1-B4-243-Tx2 08-1-B4-243-Tx4 08-1-B4-243-PM	243680	65.5%		
08-1-B4-246-PM	250042	N/A		
08-1-E2-218-Tx2	251206	N/A	N/A	
08-1-F8-128-Tx1	249468	N/A	64.2%	
08-1-F8-135-Tx1	253155	N/A		
08-1-F8-220-Tx1	256960	N/A		
08-1-F8-200-Tx2 08-1-F8-200-Tx3	250558	96.4%		
08-1-F9-55-PM-lung	259652	N/A	92.1%	
08-1-F9-111-PM-lung	251908	N/A		
08-1-F9-160-Tx2	258449	N/A		
08-1-F9-179-Tx1 08-1-F9-179-Tx3	260411	96.4%		
08-1-F9-186-PM-lung	261406	N/A		
08-1-F9-206-Tx2	251711	N/A		
08-1-F9-231-Tx2	260514	N/A		

Table 10: Summary of minimum inhibitory concentrations (MIC) for M. bovis strains collected during 2007 for 10 common antimicrobials used in the treatment or prevention of undifferentiated bovine respiratory disease.

Strain	Amp	Cef	CTC	Enr	Ery	Flor	OTC	Spec	Til	Tul
07-1-A10-21-Tx1	32	64	2	0.12	32	2	2	2	128	1
07-1-A10-148-Tx1	32	64	2	0.12	32	2	2	2	128	2
07-1-A10-148-PM-lung	32	64	2	0.12	32	2	2	2	128	4
07-1-A10-227-Tx2	32	64	2	0.12	32	2	4	2	128	4
07-1-A2-98-PM-lung	32	64	2	0.12	32	2	4	4	128	4
07-1-A8-114-Tx5	32	64	4	0.12	32	2	4	2	128	4
07-1-A8-114-PM-lung	32	64	2	0.12	32	2	4	2	128	4
07-1-A8-130-PM-joint	32	64	2	0.12	32	2	4	2	128	4
07-1-A8-130-PM-lung	32	64	2	0.12	32	2	2	2	128	8
07-1-B3-91--Tx1A	32	64	2	0.12	32	2	4	2	128	4
07-1-B3-91-Tx1B	32	64	4	0.12	32	2	4	2	128	4
07-1-B3-150-PM-lung	32	64	2	0.12	32	2	4	2	128	2
07-1-C13-211-Tx2	32	64	2	0.12	32	2	2	2	128	2
07-1-C13-211-PM-lung	32	64	2	0.12	32	2	4	2	128	2
07-1-E2-5-Tx2	32	64	2	0.12	32	2	4	2	128	2
07-1-E2-22-Tx1	32	64	2	0.12	32	2	4	2	128	4
07-1-E2-23-Control	32	64	2	0.12	32	2	2	2	128	2
07-1-E2-77-Tx1	32	64	2	0.12	32	2	4	2	128	8
07-1-E2-110-PM-lung	32	64	2	0.12	32	2	2	2	128	2
07-1-E2-144-Control	32	64	2	0.12	32	2	2	2	128	2
07-1-E2-159-Tx1A	32	64	2	0.12	32	2	4	2	128	2
07-1-E2-159-Tx1B	32	64	2	0.12	32	2	4	2	128	2
07-1-E2-159-PM-joint	32	64	4	0.12	32	2	4	2	128	2
07-1-E2-159-PM-lung	32	64	2	0.12	32	2	2	2	128	2
07-1-E2-257-Tx1A	32	64	2	0.12	32	2	4	2	128	4
07-1-E2-257-Tx1B	32	64	2	0.12	32	2	4	2	128	2
07-1-E2-257-Tx2	32	64	2	0.12	32	2	2	2	128	2
07-1-E2-257-PM-lung	32	64	4	0.12	32	2	4	2	128	2
07-1-A10-21-Tx1	32	64	2	0.12	32	2	2	2	128	1

Amp, Ampicillin; Cef, Ceftiofur; CTC, Chlortetracycline; Enr, Enrofloxicin; Ery, Erythromycin; Flor, Florfenicol; OTC, Oxytetracycline; Spec, Spectinomycin; Til, Tilmicosin; Tul, Tulathromycin.

Table 11: Summary of minimum inhibitory concentrations (MIC) for *M. bovis* strains collected during 2008 for 10 common antimicrobials used in the treatment or prevention of undifferentiated bovine respiratory disease.

	Amp	Cef	CTC	Enr	Ery	Flor	OTC	Spec	Til	Tul
08-1-B4-21-Tx1	32	64	4	0.12	32	2	4	2	128	2
08-1-B4-37-PM-Lung	32	64	2	0.12	32	2	2	2	128	8
08-1-B4-106-PM-lung	32	64	4	0.12	32	2	2	2	128	8
08-1-B4-175-Tx2	32	64	2	0.12	32	2	2	2	128	8
08-1-B4-193-Tx1	32	64	4	0.12	32	2	4	2	128	8
08-1-B4-243-Tx2	32	64	4	0.12	32	2	4	2	128	2
08-1-B4-243-Tx4	32	64	4	0.12	32	2	4	2	128	2
08-1-B4-243-PM	32	64	2	0.12	32	2	2	2	128	4
08-1-B4-246-PM	32	64	2	0.12	32	2	2	2	128	8
08-1-E2-218-Tx2	32	64	2	0.12	32	1	2	2	128	1
08-1-F8-128-Tx1	32	64	4	0.12	32	2	4	2	128	2
08-1-F8-135-Tx1	32	64	2	0.12	32	2	2	2	128	2
08-1-F8-220-Tx1	32	64	2	0.12	32	1	2	2	128	8
08-1-F8-200-Tx2	32	64	4	0.12	32	2	4	2	128	2
08-1-F8-200-Tx3	32	64	2	0.12	32	2	2	2	128	2
08-1-F9-55-PM-lung	32	64	4	0.12	32	2	4	2	128	8
08-1-F9-111-PM-lung	32	64	4	0.12	32	8	4	1	128	8
08-1-F9-160-Tx2	32	64	2	0.12	32	2	2	2	128	8
08-1-F9-179-Tx1	32	64	2	0.12	32	2	2	2	128	2
08-1-F9-179-Tx3	32	64	2	0.12	32	2	2	2	128	2
08-1-F9-186-PM-lung	32	64	4	0.12	32	2	4	1	128	2
08-1-F9-206-Tx2	32	64	2	0.12	32	2	2	2	128	2
08-1-F9-231-Tx2	32	64	4	0.12	32	2	2	2	128	2

Amp, Ampicillin; Cef, Ceftiofur; CTC, Chlortetracycline; Enr, Enrofloxicin; Ery, Erythromycin; Flor, Florfenicol; OTC, Oxytetracycline; Spec, Spectinomycin; Til, Tilmicosin; Tul, Tulathromycin.

Table 12: A summary of the overall pen-level morbidity and mortality rates compared to the direct Bovine Viral Diarrhea persistent infection exposure of the pen as determined by immunohistochemisrty.

Pen:	N	Morbidity:	Mortality:	BVD PI Status:
2007:				
A2	247	26 (10.5%)	8 (3.2%)	N
A8	247	20 (8.1%)	5 (2.0%)	N
A10	250	12 (4.8%)	3 (1.2%)	N
B3	261	48 (18.4%)	4 (1.5%)	N
B9	236	22 (9.3%)	1 (0.4%)	N
C13	256	24 (9.3%)	4 (1.6%)	Y
E2	260	44 (16.9%)	9 (3.5%)	N
2008:				
A5	269	5 (1.9%)	1 (0.3%)	N
A8	247	21 (8.5%)	8 (3.2%)	Y
B1	255	20 (7.8%)	2 (0.7%)	Y
B4	254	17 (6.7%)	8 (3.1%)	N
B8	255	5 (2.0%)	3 (1.1%)	N
E2	248	22 (8.9%)	1 (0.4%)	N
F8	255	18 (7.1%)	2 (0.7%)	N
F9	252	29 (11.5%)	8 (3.2%)	Y